IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	Examiner: Haddad, Maher M.
Avi ASHKENAZI, et al.	)	Group Art Unit: 1644
Application Serial No. 10/767,374	)	Confirmation No. 4761
Filed: January 29, 2004	)	Attorney's Docket No. 39780-1216 R1C1D1
For: **PRO362 POLYPEPTIDES**	)	**Customer No. 35489**

SECOND DECLARATION OF MENNO VAN LOOKEREN CAMPAGNE, Ph.D. UNDER 37 C.F.R. § 1.132

I, Menno van Lookeren, Ph.D., declare and say as follows:

1. I obtained a masters degree in neuropharmacology from the State University of Utrecht, Utrecht, the Netherlands in 1987. In 1991, I was awarded a Ph.D. at the Rudolf Magnus Institute of Pharmacology, State University of Utrecht, Utrecht, the Netherlands.

2. Between 1991 and 1997 I worked as a post-doctoral fellow, first at the Netherlands Institute of Brain Research, Amsterdam, the Netherlands, and later at Hoffmann-La Roche, Department of Neuroscience, Basel, Switzerland. From 1997 to 1999 I was employed as a visiting scientist at the Department of Cardiovascular Research of Genentech, Inc., South San Francisco, CA. I am currently a scientist at the Department of Immunology of Genentech, Inc.

3. My Curriculum Vitae, including a list of my publications, is attached to and forms part of this Declaration (Exhibit A).

4. My current responsibilities include strategic leadership for initiation and advancement of discovery programs in immunology. As part of these responsibilities, I am responsible for the identification and functional characterization of novel molecules expressed on myeloid cells and for developing therapeutic entities for the treatment of inflammatory and autoimmune diseases.

5. Experiments to evaluate the expression of PRO362 in various types of tissues by in situ hybridization were performed in my laboratory either personally by myself or under my supervision.

6. For in situ hybridization, tissues were fixed (4% formalin), paraffin-embedded, sectioned (3-5 μm thick), deparaffinized, deproteinated (20 μg/ml) with proteinase K (15 minutes at 37°C), and processed for in situ hybridization. Probes were produced by PCR. Primers included T7 or T3 RNA polymerase initiation sites to allow for in vitro transcription of sense or antisense probes from the amplified products. ^{33}P-UTP labeled sense and antisense probes were hybridized overnight (55°C), washed (0.1 X SSC for 2 hours at 55°C), dipped in NBT2 nuclear track emulsion (Eastman Kodak, Rochester, NY), exposed (4-6 weeks at 4°C), and developed and counterstained with hematoxylin and eosin. Representative paired bright and darkfield images are typically shown.

7. Immunohistochemical staining was performed on 5 mm thick frozen sections using a DAKO Autostainer. Endogenous peroxidase activity was blocked with Kirkegaard and Perry Blocking Solution (1:10, 4 minutes at 20C). 10% NGS in TBS/0.05% Tween-20 (DAKO) was used for dilution and blocking. MAb 4F722.2 anti-PRO362 or mouse IgG was used at 0.13 mg/ml. Biotinylated goat anti-mouse IgG (Vector Labs, Burlingame, CA) was used at 1:200 and detected with Vector Labs Standard ABC Elite Kit (Vector Labs, Burlingame, CA). Slides were developed using Pierce metal-enhanced diaminobenzidine (Pierce Chemicals, Rockford, IL).

8. Expression was examined in a wide variety of tissues and cell types from humans and other mammals. Normal human adult tissues that were examined included tonsil, lymph node, spleen, kidney, urinary bladder, lung, heart, aorta, coronary artery, liver, gall bladder, prostate, stomach, small intestine, colon, pancreas, thyroid gland, skin, adrenal gland, placenta, uterus, ovary, testis, retina, and brain (cerebellum, brainstem, cerebral cortex). Normal human fetal tissues including E12-E16 week-old brain, spleen, bowel and thyroid were also tested. In addition, expression was investigated in murine liver. Inflamed tissues examined by in situ hybridization included tissues with chronic inflammatory disease such as lungs with chronic asthma, chronic bronchopneumonia, chronic bronchitis/chronic obstructive pulmonary disease, kidneys with chronic lymphocytic interstitial nephritis, and livers with chronic inflammation and cirrhosis due to chronic hepatitis C infection, autoimmune hepatitis or alcoholic cirrhosis.

9. As attested by the results of in situ hybridization experiments, PRO362 mRNA was found to be present in mouse liver frozen sections (Figure 1), human liver frozen sections (Figure 2) and a number of tissue macrophage-like cells, including colon macrophages (Figure 3A), Kupffer cells (Figure 3B), adrenal macrophages (Figure 3C), Hofbauer cells (Figure 3D), synovial cells (Figure 4), alveolar macrophages, resident macrophages in the intestinal lamina propria and interstitial macrophages in many tissues. PRO362 was also significantly expressed in brain microglia.

10. We studied the expression pattern of PRO362 mRNA in synovium obtained from patients with rheumatoid arthritis, osteoarthritis and degenerative bone disease. High expression of PRO362 mRNA was found in synovial cells obtained from a patient with osteoarthritis (Fig. 5A-D). Synovial cells in the superficial layers had the highest expression of PRO362 mRNA (Fig. 5D).

11. In order to quantitatively determine PRO362 mRNA expression levels in inflammatory bowel disease (IBD) syndrome, PRO362 mRNA was extracted from colon tissue obtained from patients with ulcerative colitis, Crohn's disease or from patients with no manifestation of IBD. Real time PCR was performed using primers specific for PRO362, to measure relative expression levels. Expression levels of PRO362 mRNA were 16 fold higher in a patient with ulcerative colitis and, 5 fold higher in a patient with Crohn's disease, as compared to control tissue (Fig. 6A). Similarly, relative RNA equivalents were determined in lung tissue and was found to be highest in tissue from a patient with chronic occlusive pulmonary disease (COPD: 14 fold over normal) and was not significantly different from normal in a patient with asthma (Fig. 6B).

12. Using immunohistochemistry, expression of PRO362 protein was found on macrophages in a number of different tissues. Frozen sections prepared from CHO cells stably expressing PRO362 show membrane localization of PRO362 (Fig. 7A). PRO362 protein was found in alveolar macrophages (Fig. 7B), histiocytes in the lamina propria of the small intestine (Fig. 7C), Hofbauer cells in the placenta (Fig. 7D), macrophagesin the adrenal gland (Fig. 7E) and Kupffer cells in the liver (Fig. 7F).

13. Polyclonal antibody 6F1 was used to study expression of the PRO362 protein in frozen sections of human synovium obtained from a patient with rheumatoid arthritis. PRO362 protein was expressed in a subset of synovial cells (20-40%) and in tissue macrophages in the synovium (Fig. 8A-C). These cells were, most likely, type A macrophage-like synovial cells. Staining was absent in control synovium (Fig. 8D).

14. Atherosclerotic plaques contained a high number of macrophages or macrophage-foam cells that adhered tightly to the luminal wall of the aorta. Considering a role for PRO362 in macrophage-endothelium adhesion, the expression of PRO362 in atherosclerotic plaques was studied. Alternate sections of plaques were stained with anti CD63 antibodies (Fig. 9A and B) or anti-PRO362 antibodies (Fig.9C and D). Overlapping staining patterns of anti-CD63 and anti-PRO362 was found on foam cells aligning the vessel wall indicating a role for PRO362 in atherosclerosis.

15. It is my considered scientific opinion that the increased expression of PRO362 mRNA and protein in various inflammatory diseases, as discussed in paragraphs 9-14 above, makes it a good target for the diagnosis of inflammatory diseases, and that such diagnosis can be carried out, for example, using anti-PRO362 antibodies, specifically binding PRO362.

16. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

Menno van Lookeren Campagne, Ph.D.

05.01. 2007
Date

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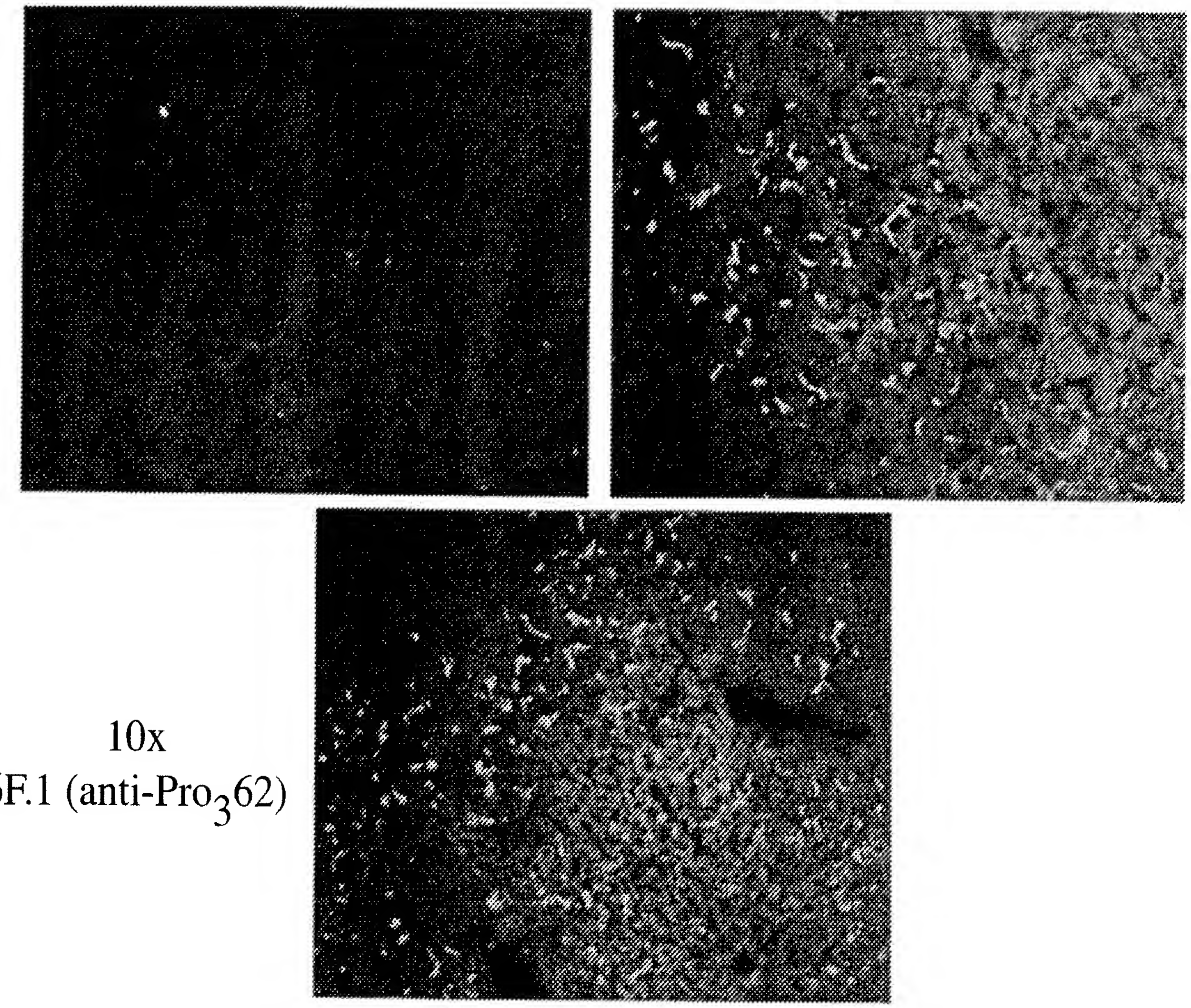


FIG. 1

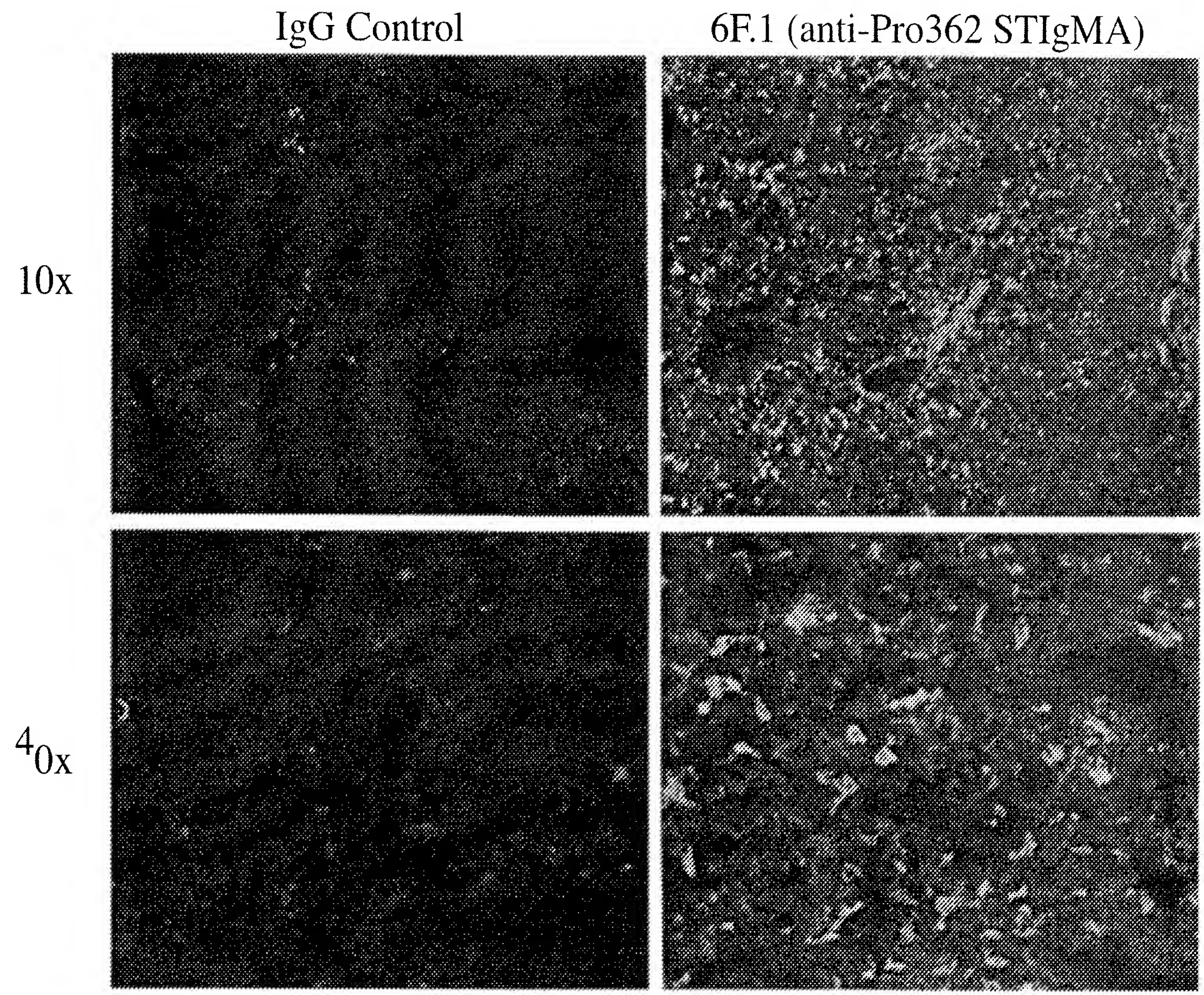


FIG. 2

FIG. 3 A-D

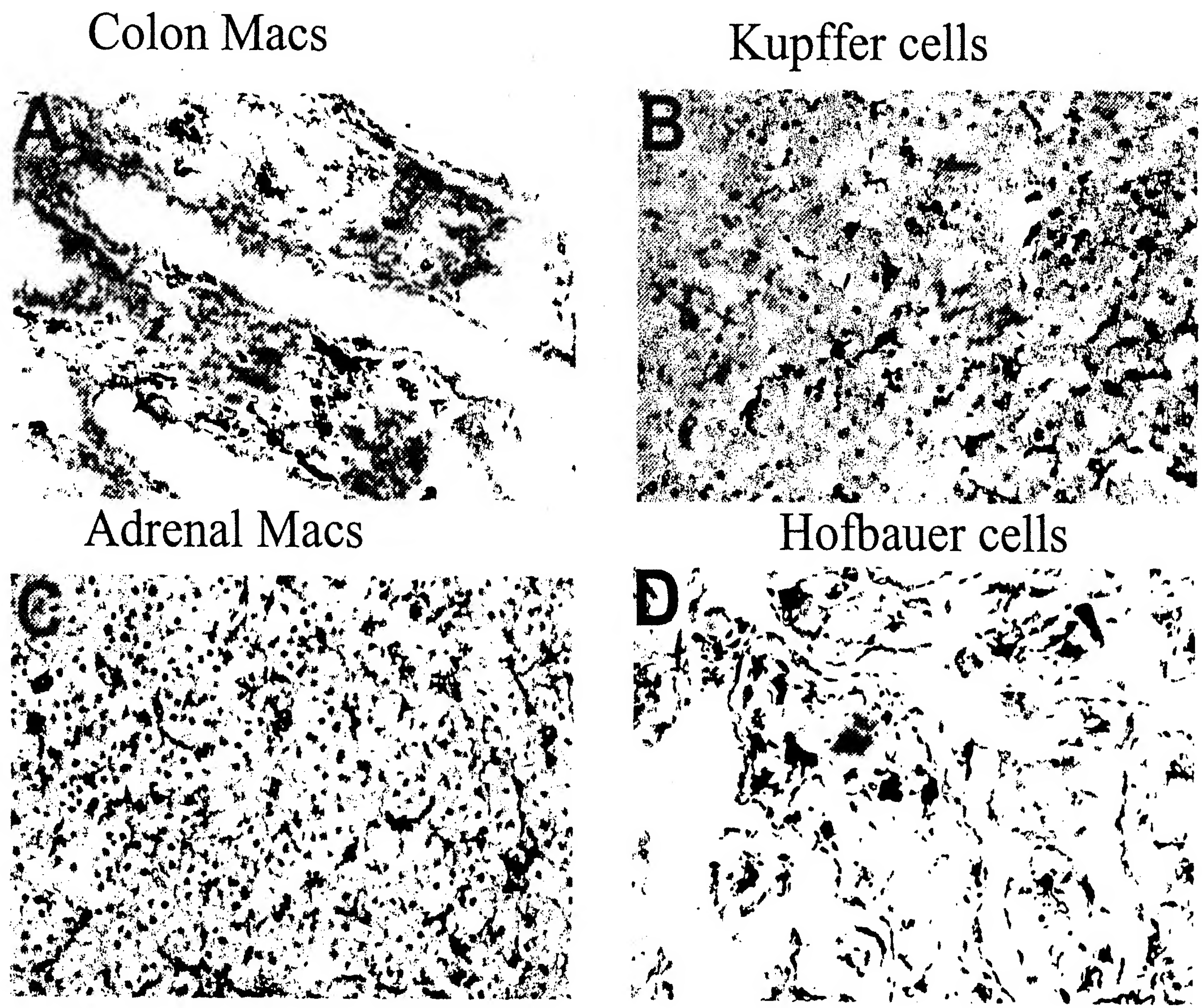
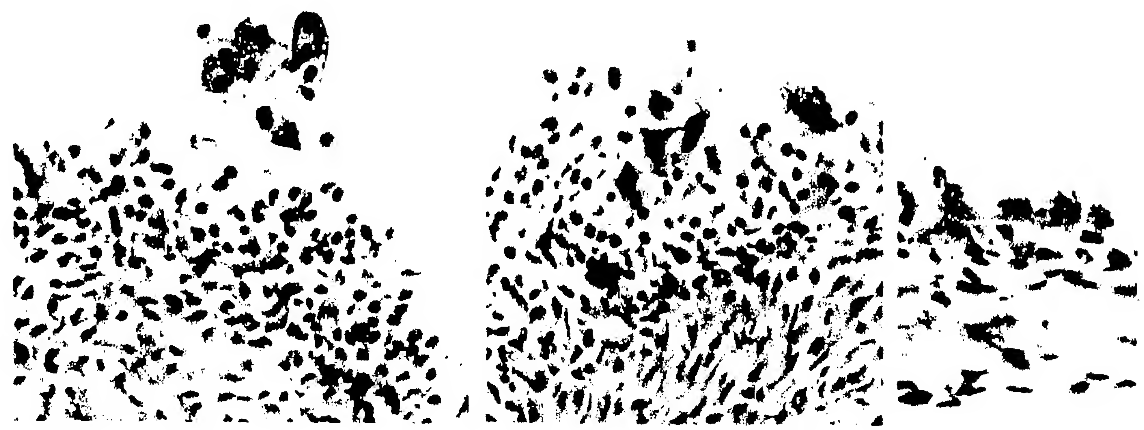


FIG.4

Synovial cells



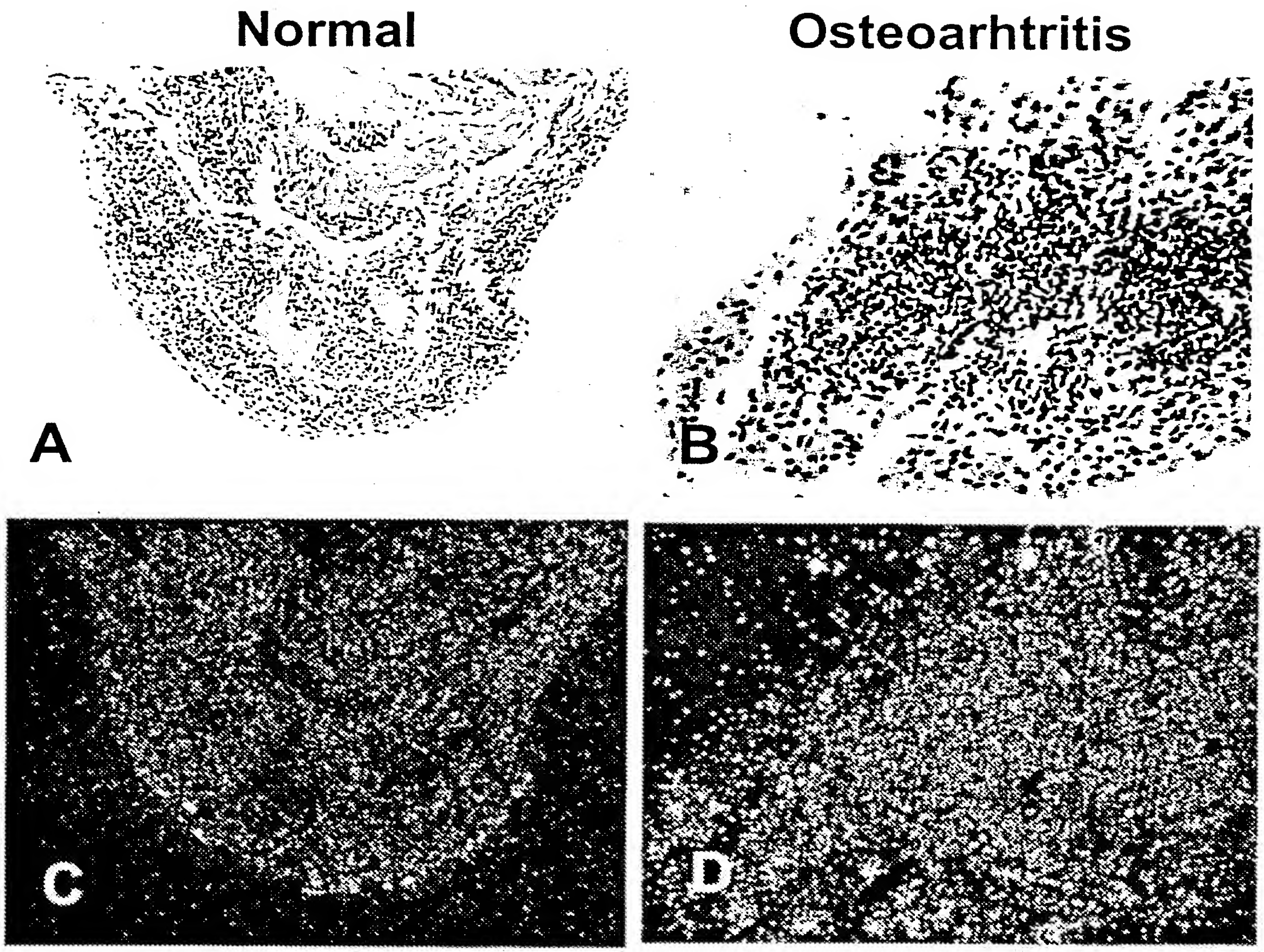
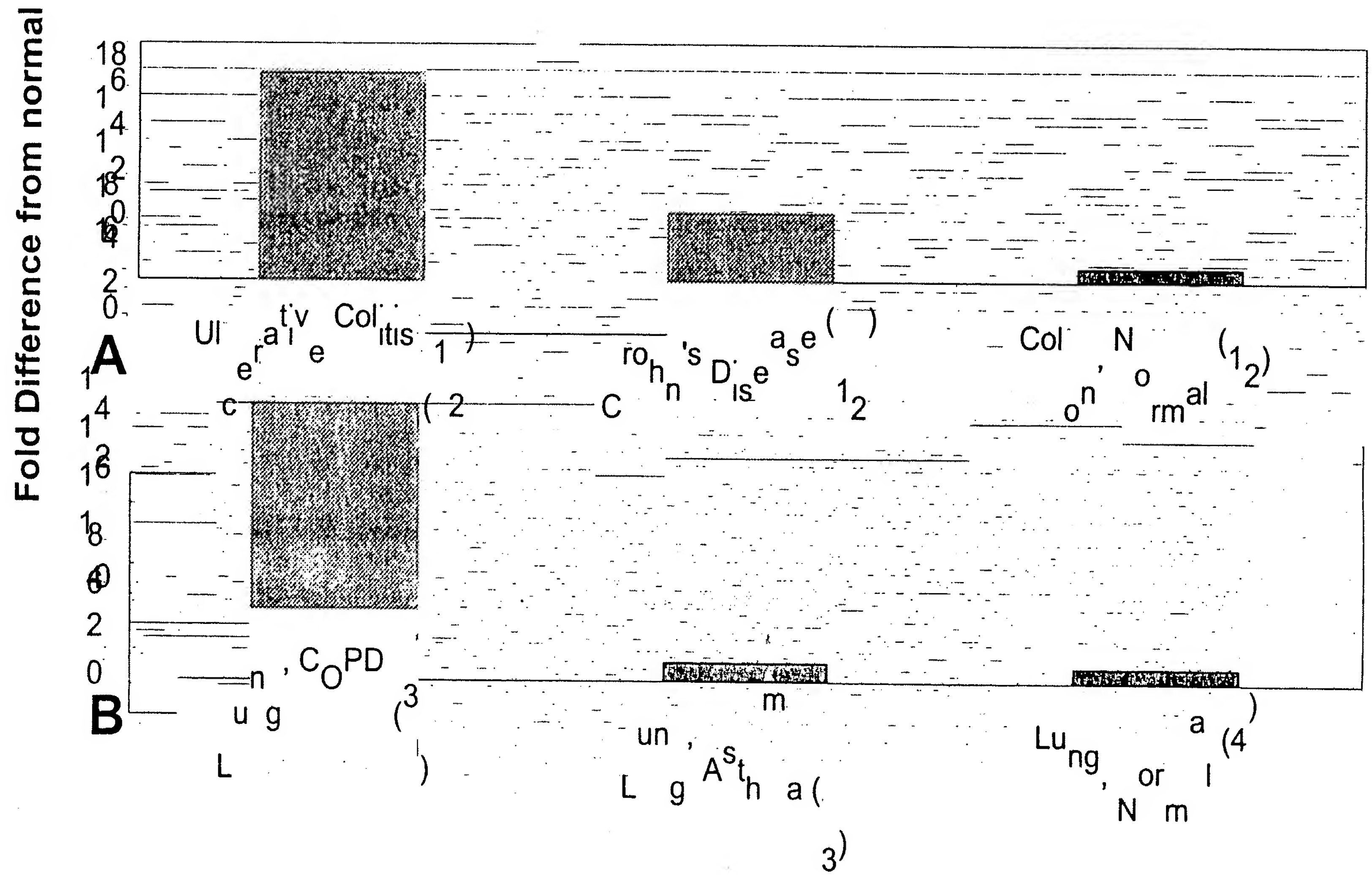


FIG. 5 A-D



A B
G 6

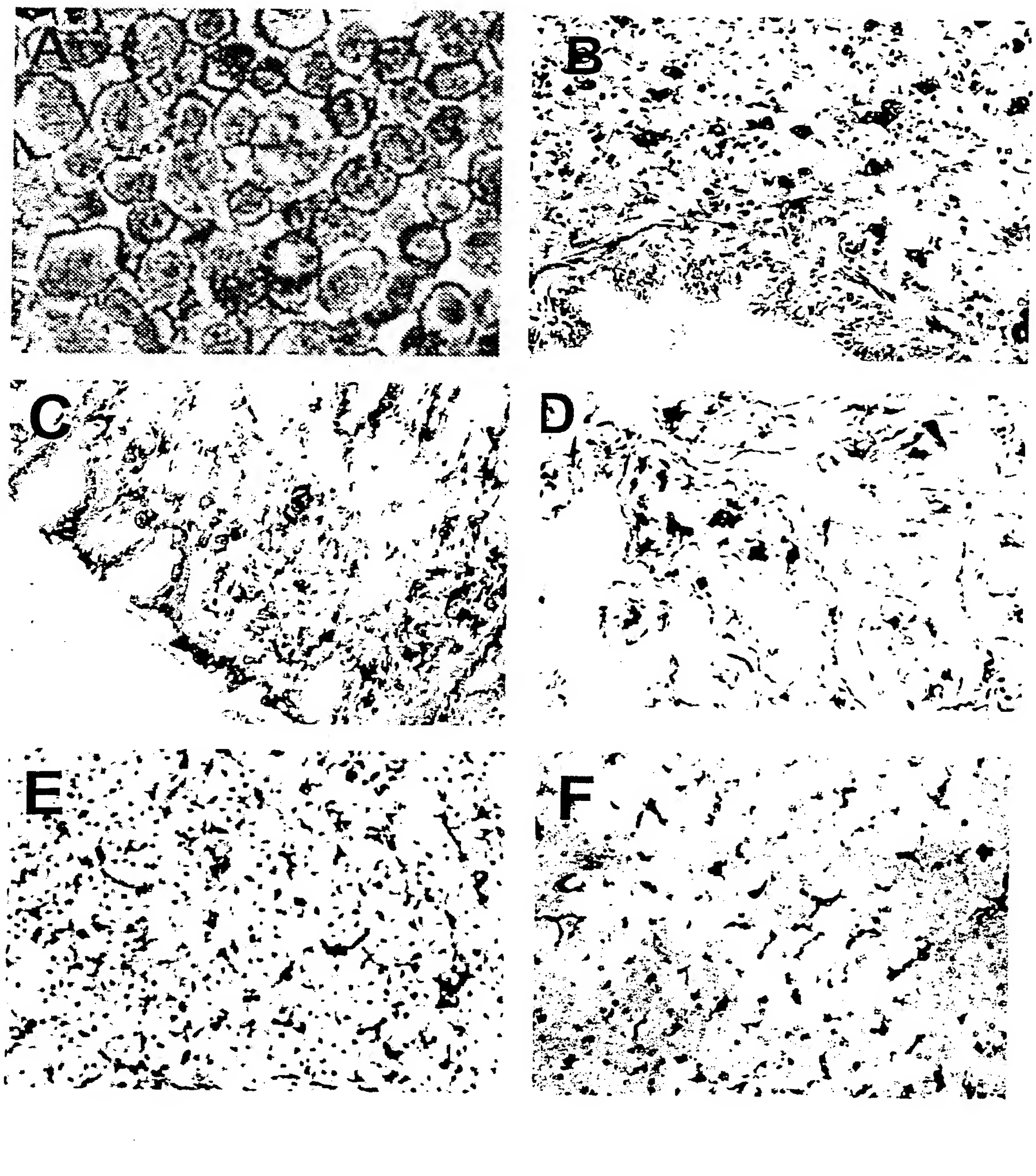


FIG. 7 A-F

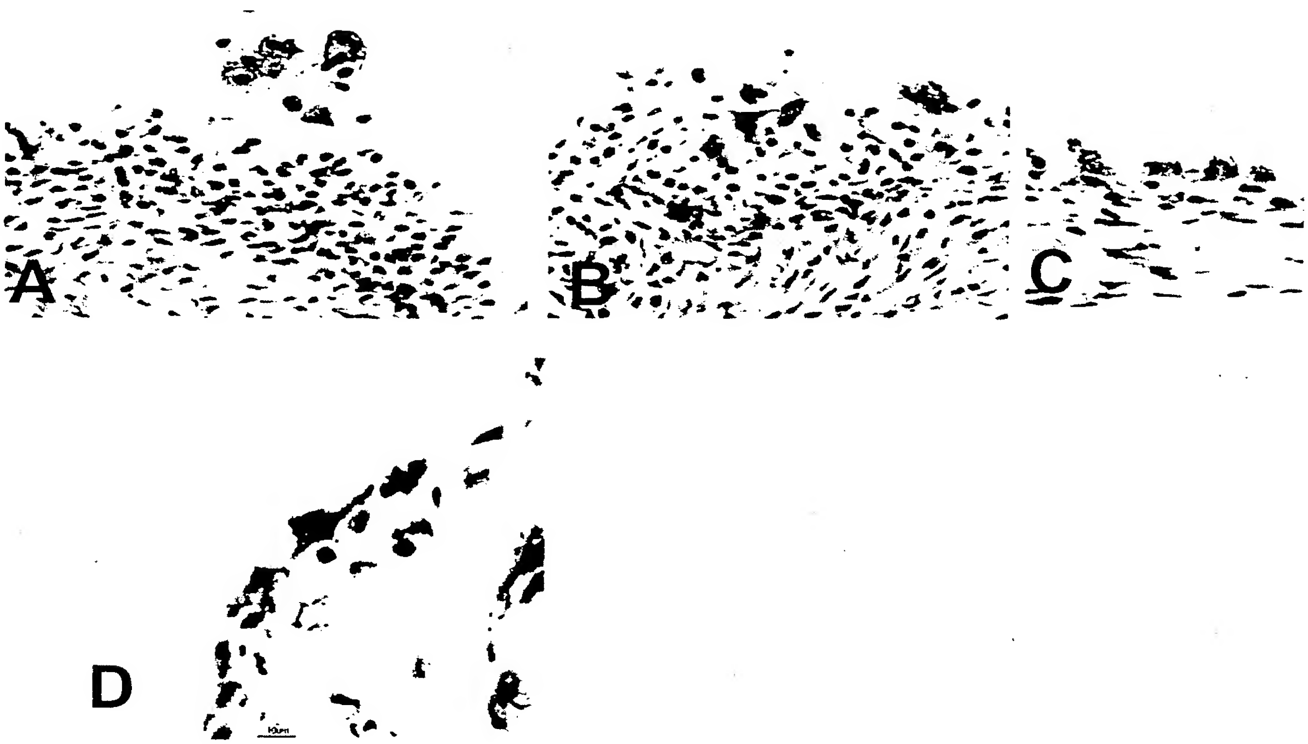


FIG. 8 A-D

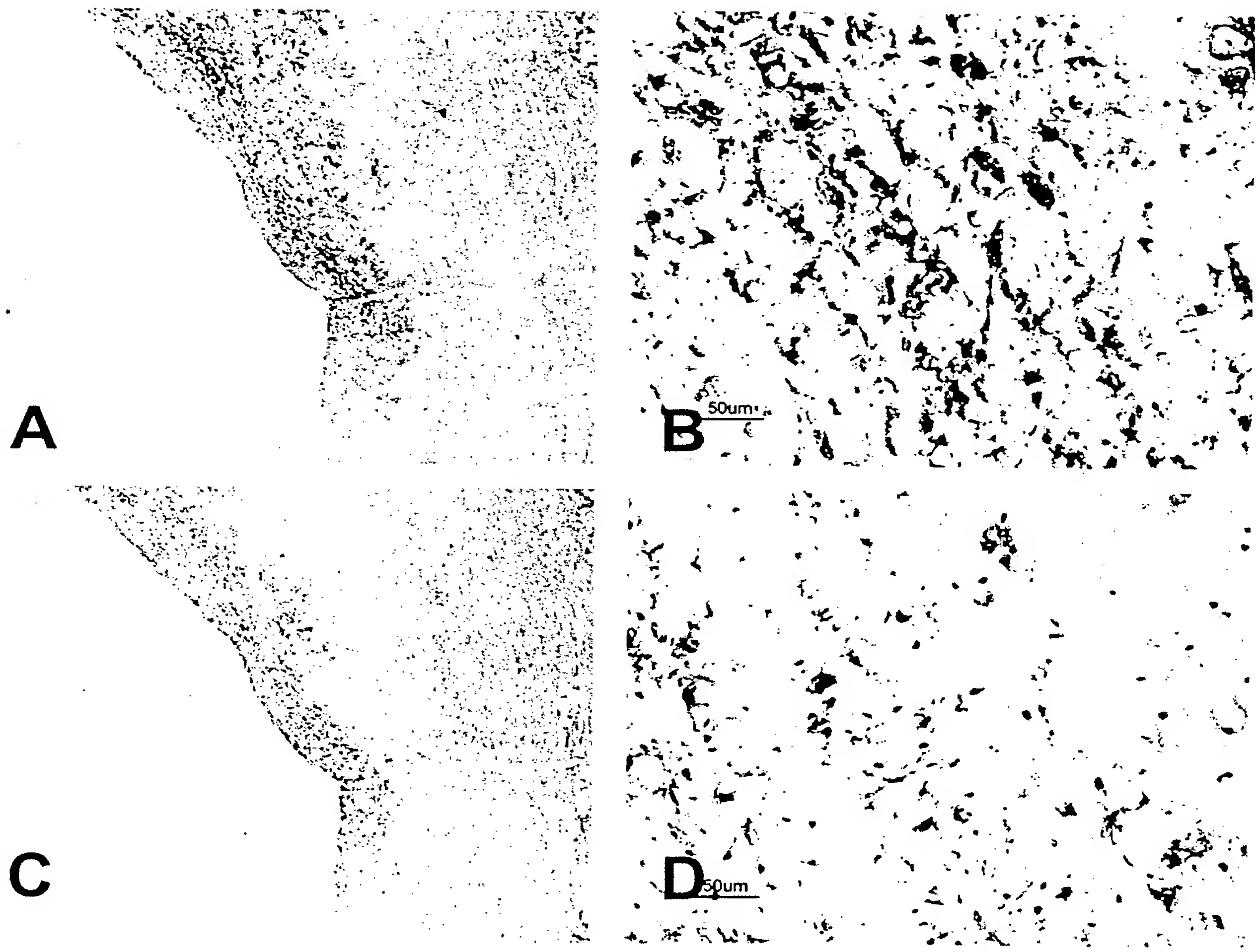


FIG. 9 A-D